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Amyloid $\beta_{1–42}$ peptide alters the gating of human and mouse α-bungarotoxin-sensitive nicotinic receptors

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The β-amyloid$_{1–42}$ peptide ($A\beta_{1–42}$), a major constituent of the Alzheimer's disease amyloid plaque, specifically binds to the neuronal α-bungarotoxin (α-BuTx)-sensitive α7 nicotinic acetylcholine receptor (α7 nAChR). Accordingly, $A\beta_{1–42}$ interferes with the function of α7 nAChRs in chick and rodent neurons. To gain insights into the human disease, we studied the action of $A\beta_{1–42}$ on human α7 nAChRs expressed in *Xenopus* oocytes. In voltage-clamped oocytes expressing the wild-type receptor, $A\beta_{1–42}$ blocked ACh-evoked currents. The block was non-competitive, required over 100 s to develop and was partially reversible. In oocytes expressing the mutant L248T receptor, $A\beta_{1–42}$ activated methyllycaconitine-sensitive currents in a dose-dependent manner. Peptide-evoked unitary events, recorded in outside-out patches, showed single-channel conductances and open duration comparable to ACh-evoked events. $A\beta_{1–42}$ had no effect on the currents evoked by glutamate, GABA or glycine in oocytes expressing human or mouse receptors for these transmitters. Muscle nAChRs are also α-BuTx-sensitive and we therefore investigated whether they respond to $A\beta_{1–42}$. In human kidney BOSC 23 cells expressing the fetal or adult mouse muscle nAChRs, $A\beta_{1–42}$ blocked ACh-evoked whole-cell currents, accelerating their decay. Outside-out single-channel recordings showed that the block was due to a reduced channel open probability and enhanced block upon ACh application. We also report that the inverse peptide $A\beta_{42–1}$, but not $A\beta_{40–1}$, partially mimicked the effects of the physiological $A\beta_{1–42}$ peptide. Possible implications for degenerative neuronal and muscular diseases are discussed.

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder whose histological hallmark is the presence of amyloid plaques in the limbic and cerebral cortices (for review, see Selkoe, 1994). Although multiple neural systems are affected, a key feature of the neurodegenerative process is the loss of cholinergic neurons as well as nicotinic acetylcholine receptors (nAChRs) throughout the brain (Guan *et al.* 2000; Nordberg, 2001). The major constituent of the amyloid plaques is a 42-amino-acid β-amyloid peptide ($A\beta_{1–42}$), derived from the proteolytic cleavage of the amyloid precursor protein, which is present in almost all tissues and whose physiological functions are still unknown (Selkoe, 1994, 2001).

$A\beta_{1–42}$ has recently been reported to bind specifically and with picomolar affinity to the neuronal nAChR containing the α7 subunit (α7 nAChR) (Wang *et al.* 2000*a*,*b*). The binding affinity of $A\beta_{1–42}$ to α7 nAChRs appears to be at least 1000-fold higher than that of the specific blockers α-bungarotoxin (α-BuTx) and methyllycaconitine (MLA) (Wang *et al.* 2000*a*), whereas the binding affinity of $A\beta_{1–42}$ for α-BuTx-insensitive neuronal nicotinic receptors, i.e. receptors that do not contain the α7 subunit, is much smaller (Wang *et al.* 2000*b*). Consistent with these data, $A\beta_{1–42}$ functionally blocks the ACh-evoked current responses in rat hippocampal slices (Pettit *et al.* 2001). In cultured mouse hippocampal neurons and chick ciliary ganglion nerve cells the block appears to be specific for α7 nAChRs, with little, if any, effect on α-BuTx-insensitive nAChRs (Liu *et al.* 2001). A small, slowly developing block of rat α7 nAChRs expressed in *Xenopus* oocytes has also been described (Tozaki *et al.* 2002). At complete variance with all these pieces of evidence, picomolar concentrations of $A\beta_{1–42}$ have been reported to activate rat α7 nAChRs expressed in *Xenopus* oocytes (Dineley *et al.* 2002), although only upon the very first exposure of the oocyte to the amyloid peptide. No current

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activation was reported for rat hippocampal neurones exposed to similar $A\beta_{1–42}$ concentrations (Liu *et al.* 2001). A more robust current response has been described for the rat $\alpha7$ nAChR carrying a point mutation in the pore-forming region (Dineley *et al.* 2002). The latter observation is in line with the behaviour of several antagonists of chick and human wild-type (WT) $\alpha7$ nAChRs, which become agonists of the mutant receptors carrying that particular threonine-for-leucine substitution (L247T in chick, L250T in rat and mouse, L248T in human) (Palma *et al.* 1996, 1998, 1999; Maggi *et al.* 1999; Fucile *et al.* 2000, 2002). It is therefore quite likely that $A\beta_{1–42}$-induced activation of the mutated nAChR accounts for the Ca^{2+}-induced activation of the mitogen-activated protein kinase (MAPK) pathway described in mice heterozygous for the L250T $\alpha7$ nAChR allele (Dineley *et al.* 2001). Activation of MAPK is required for contextual and spatial memory formation in mammals (Atkins *et al.* 1998), which processes are impaired in AD patients. Thus, assessing the ability of the $A\beta_{1–42}$ peptide to activate human $\alpha7$ nAChRs may provide clues to the physiological and/or pathological relevance of the $A\beta_{1–42}$–$\alpha7$ nAChR interaction to AD. Since no functional data is available for human $\alpha7$ nAChRs, in this paper we investigated the effects of $A\beta_{1–42}$ on human WT and L248T $\alpha7$ nAChRs expressed in *Xenopus* oocytes.

Additional insights into the physio-pathological importance of the interaction between $A\beta_{1–42}$ and nAChRs may come from a different disease, inclusion body myositis (IBM), which represents the most common myopathy after 50 years of age. It is characterised by the presence of plaques, within muscle fibres, where 'AD characteristic' proteins, such as $A\beta_{1–42}$ and presenilin-1, are accumulated (reviewed in Askanas & Engel, 1998), together with the end-plate nAChR. To date, IBM appears to be the only non-neuronal progressive disease caused by $A\beta$ deposition (Sugarman *et al.* 2002). Moreover, both the fetal and adult forms of muscle nAChRs (γ- and ϵ-nAChRs, respectively) share with the $\alpha7$ nAChR the sensitivity to α-BuTx, and could thus possibly become targets for $A\beta_{1–42}$ as well. Indeed, block of the *Torpedo* nAChR by $A\beta_{1–42}$ has been reported (Tozaki *et al.* 2002). Furthermore, $A\beta_{1–42}$ content is elevated in the muscle of AD patients (Kuo *et al.* 2000*b*). These considerations prompted us to investigate whether $A\beta_{1–42}$ also modulates the functional properties of mammalian α-BuTx-sensitive muscle nAChRs expressed by transient transfection in human kidney BOSC 23 cells.

METHODS

Expression of nAChRs in oocytes and BOSC 23 cells

Recombinant DNA plasmids encoding human WT $\alpha7$ (gift from Janssen, Belgium) and L248T $\alpha7$ neuronal nicotinic subunits in the pcDNA3 vector, or the human GluR1 subunit (flip-splice variant) in the pCEP4 expression vector were intranuclearly injected into stage V–VI oocytes (2 ng cDNA in 10 nl buffer). Preparation of oocytes and nuclear injection procedures were as previously detailed (Palma *et al.* 1996). Oocytes were collected under anaesthesia from frogs that were humanely killed after the final collection. In other experiments, oocytes were injected with membranes extracted from mouse cortex, according to procedures described elsewhere (Miledi *et al.* 2002). Oocytes were used for electrophysiological determinations 1–4 days after injection. Full length cDNAs in SV-40-based pSM expression vector coding for the $\alpha1, \beta, \gamma$ and δ (γ-nAChR) or the $\alpha1, \beta, \epsilon$ and δ (ϵ-nAChR) subunits (obtained from Dr J. Patrick, Baylor College of Medicine, Houston, TX, USA; 0.2 μg each per 35-mm dish) were transiently transfected in human kidney BOSC 23 cells (ATCC) using a Ca^{2+}-phosphate method, as previously described (Fucile *et al.* 1996). The cell line BOSC 23 was maintained in culture in Dulbecco's modified Eagle's medium (Euroclone, UK), supplemented with 10 % calf serum (Euroclone). Cells were washed twice 8–12 h after the start of transfection and used for experiments 36–48 h after transfection.

Voltage-clamp recordings and analysis

Membrane currents were recorded in the voltage-clamp mode using two microelectrodes filled with 3 M KCl, at controlled room temperature (20–21 °C). The oocytes were placed in a recording chamber (0.1 ml) continuously superfused (12 ml min^{-1}) with oocyte Ringer solution. Throughout the experiments, oocyte membrane potential was maintained at −60 mV, except when otherwise indicated. Multiple ACh applications to the same oocyte were performed with at least 3 min intervals. Drugs, dissolved in oocyte Ringer solution, were applied by superfusion, using electromagnetic valves (BioLogic, France) to achieve solution exchange. Currents were digitised at 50–200 Hz (Digidata 1200 analog-to-digital converter, Axon Instruments, USA) and analysed off-line using pClamp 6.0.2 routines (Axon Instruments), as detailed in Palma *et al.* (1996). The ACh concentration yielding half-maximal current response (EC_{50}) or inhibition (IC_{50}) and the Hill coefficient (n_H) were obtained as previously reported (Palma *et al.* 1996).

Patch-clamp recordings in oocytes and BOSC 23 cells

Outside-out patch-clamp recordings were performed on oocytes whose vitelline membrane had been mechanically removed after exposure to a hypertonic solution for 10–20 min, as previously described (Methfessel *et al.* 1986), using patch pipettes with narrow tips, in order to avoid the occurrence of stretch-activated channels (Methfessel *et al.* 1986). An Axopatch 200B amplifier (Axon Instruments) was used for recordings. Excised patches were continuously superfused with oocyte Ringer solution (supplemented with ammonia, when appropriate, to the same final concentration as $A\beta$-containing solutions) or agonist-containing solutions via independent tubes, positioned 50–100 μm from the electrode tip and connected to a gravity-driven fast-exchanging perfusion system (RSC 200, BioLogic). This system was also used in all the experiments with BOSC 23 cells. Unless otherwise indicated, whole-cell and outside-out recordings were performed at a membrane holding potential of −70 mV for BOSC 23 cells and −50 mV for oocytes. Whole-cell currents were digitised at 500 Hz and analysed with pCLAMP programs (pCLAMP 8, Axon Instruments). The time to half-decay ($T_{0.5}$), defined as the time taken for the current to decrease from peak to half-peak value, was used to estimate the rate of current decay. Single-channel currents were recorded in the cell-attached or outside-out configuration. Data were sampled at 10 kHz and analysed after Gaussian digital filtering at 2 kHz, using

a threshold-crossing method by pCLAMP 6.0.2 routines, as previously detailed (Fucile *et al.* 1996). Total channel open probability (NP_{op}) was estimated as the percentage of time spent in the open state, taking into account multiple openings. Once exposed to $A\beta_{1-42}$, cells were discarded. Statistical significance was accepted for $P < 0.05$.

Drugs, chemicals and solutions

Analytical grade reagents were purchased from Sigma (USA), except for methyllycaconitine (MLA, RBI, USA). Amyloid β peptides were obtained from different companies: $A\beta_{1-42}$ from Alexis (USA), Bachem (CH) or Sigma; $A\beta_{42-1}$ from Bachem; $A\beta_{40-1}$ from Sigma. Peptides were dissolved in water ($A\beta_{40-1}$), 0.1 % ammonia (Bachem $A\beta_{1-42}$ and $A\beta_{42-1}$), 100 % DMSO (Alexis $A\beta_{1-42}$) or 100 mM acetic acid (Sigma $A\beta_{1-42}$) at concentrations ranging from 0.2 to 2 mM and stored in aliquots at −20 °C until use. As in other studies (Liu *et al.* 2001; Pettit *et al.* 2001), no attempts were made to control the aggregation state of the peptide. However, $A\beta$ peptides were diluted to the final concentration just prior to use, which minimises aggregation. Different lots of $A\beta_{1-42}$ from each source were used. Two lots of Bachem $A\beta_{1-42}$ were poorly effective on nAChRs, as previously reported for $A\beta_{1-40}$ from the same company (Simmons *et al.* 1994). Oocyte Ringer solution contained (mM): NaCl 82.5, KCl 2.5, $CaCl_2$ 2.5, $MgCl_2$ 1, Hepes/NaOH 5 (pH 7.4). The patch pipettes for outside-out recordings in oocytes were filled with a solution containing (mM): CsF 80, EGTA 5, Hepes/CsOH 5; pH 7.4. BOSC 23 cells were bathed in a salt solution composed of (mM): NaCl 140, KCl 2.8, $CaCl_2$ 2, $MgCl_2$ 2, Hepes/NaOH 10, glucose 10 (pH 7.3)(plus ammonia 0.0001 % or DMSO 0.05 %, if required). The patch pipettes for recordings in BOSC 23 cells were filled with the above saline for cell-attached recordings, or with an internal solution containing (mM): CsCl 145, BAPTA 5, Hepes/CsOH 10, Mg-ATP 2 (pH 7.3) for whole-cell and outside-out recordings.

RESULTS

$A\beta_{1-42}$ blocks WT α7 nAChRs

The main aim of this paper was to investigate the functional modulation of the human α7 nAChR upon exposure to the $A\beta_{1-42}$ peptide. The current evoked by ACh (I_{ACh}) was measured in oocytes expressing WT α7 nAChRs, the best characterised expression system for this receptor. In 13 oocytes tested (three donors, 13/3), $A\beta_{1-42}$ at concentrations ranging from 10 pM to 1 μM was unable to elicit current responses (Fig. 1*A*). Each dose of $A\beta_{1-42}$ was applied, in random order, for 2–10 s, followed by a 5 min wash-out. All the oocytes were responsive to ACh (100 μM, the EC_{50} for this preparation, see below) (e.g. Fig. 1*A*), which was only applied at the end of the trials with amyloid peptide, to avoid artefacts due to solution

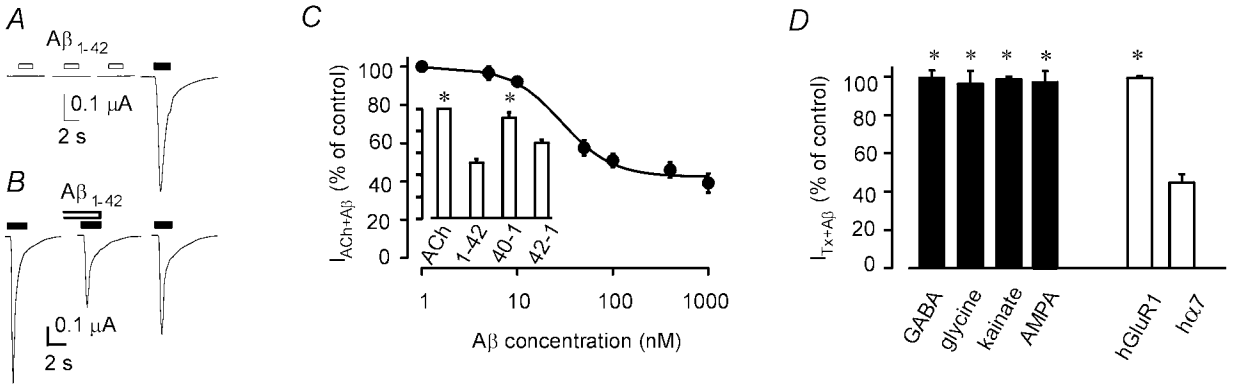

Figure 1. Block of WT α7 nAChRs by $A\beta_{1-42}$

A, $A\beta_{1-42}$ at concentrations of 0.01, 0.1 and 10 nM (open bars) fails to elicit current responses in an oocyte sensitive to ACh (100 μM, filled bar). Traces representative of 8 experiments, where $A\beta_{1-42}$ concentrations were applied in random order. *B,* inward currents evoked by 100 μM ACh (filled bars) in an oocyte expressing human WT α7 nAChRs in standard solution (left), after 180 s preincubation with 100 nM of $A\beta_{1-42}$ (open bar, middle), and 35 min after wash-out (right). Note the incomplete recovery of I_{ACh}. *C,* block of I_{ACh} by increasing doses of $A\beta_{1-42}$. In each of 5 oocytes, currents evoked by ACh (100 μM) plus $A\beta_{1-42}$ after 180 s preincubation with the peptide were normalised to the response to ACh alone (−0.67 ± 0.11 μA, 5/1). Best fitting to the Hill equation yielded an IC_{50} of 90 nM. Inset, histogram representing the effects of 100 nM $A\beta_{1-42}$, $A\beta_{42-1}$ and $A\beta_{40-1}$. I_{ACh} was measured after 180 s incubation with the peptides and normalised to the control value in each cell (bar labelled ACh). Bars represent mean ± S.E.M. of 5–11 oocytes (3 donors). * Statistically not different from control (Student's *t* test, $P = 0.2$). ACh concentration, 100 μM. Note the reduced effect of $A\beta_{42-1}$ as compared to $A\beta_{1-42}$ and the ineffectiveness of $A\beta_{40-1}$. *D,* $A\beta_{1-42}$ (0.4 μM for 180 s) is unable to block currents evoked by AMPA (50 μM plus cyclothiazide 50 μM), kainate (200 μM), GABA (1 mM) or glycine (1 mM), while blocking α7 nAChRs in *Xenopus* oocytes. Filled bars represent mean ± S.E.M. from 4 oocytes injected with mouse brain membranes. Inward current amplitude was: 0.08–0.68 μA (GABA); 0.01–0.03 μA (glycine); 0.09–0.13 μA (kainate); 0.3–0.44 μA (AMPA). Open bars represent mean ± S.E.M. of 5 oocytes injected with cDNAs encoding human *homomeric* GluR1 or α7 nAChRs, as indicated. Current ranged from 0.2–0.4 μA (hGluR1, activated by AMPA as above), from 0.2–1.0 μA (α7 nAChR, ACh 100 μM). Holding potential was –80 mV; the protocol of $A\beta_{1-42}$ treatment was as in *B.* * Statistically not different from control (Student's *t* test, $P > 0.23$).

contamination. The inhibitory action of $A\beta_{1–42}$ was investigated using the same peptide concentration (100 nM) as used by other investigators (Liu *et al.* 2001; Pettit *et al.* 2001; Dineley *et al.* 2002; Tozaki *et al.* 2002). I_{ACh} did not change when $A\beta_{1–42}$ was co-applied with ACh (data not shown). However, after 180 s exposures to $A\beta_{1–42}$, the amplitude of the current evoked by ACh (100 μM) was markedly reduced, in agreement with previous reports (Liu *et al.* 2001; Pettit *et al.* 2001; Dineley *et al.* 2002; Tozaki *et al.* 2002). In the 16 oocytes tested from four donors (16/4), the amplitude of I_{ACh} was -0.55 ± 0.18 μA, i.e. 51 ± 8 % (mean ± S.E.M.) of the control (Fig. 1*B* and *C*). A comparable reduction of I_{ACh} was observed at test potentials of –100 mV and –60 mV (4/2), indicating that the effect of $A\beta_{1–42}$ was voltage independent in this range (data not shown). $A\beta_{1–42}$ exerted no effect on current decay, with similar values of $T_{0.5}$ measured before and during treatment (0.53 ± 0.11 and 0.57 ± 0.13 s, respectively). The block was poorly reversible, as 35 min after wash-out of $A\beta_{1–42}$, I_{ACh} was 75.7 ± 3 % of control (e.g. Fig. 1*B*). To test whether the lack of full recovery was due to voltage-dependent interactions between $A\beta_{1–42}$ and α7 nAChRs, the oocyte holding potential was stepped to +30 mV for 10 s during $A\beta_{1–42}$ wash-out. However, recovery was not accelerated, I_{ACh} amplitude being 71 % of control 25 min after peptide withdrawal (2/2).

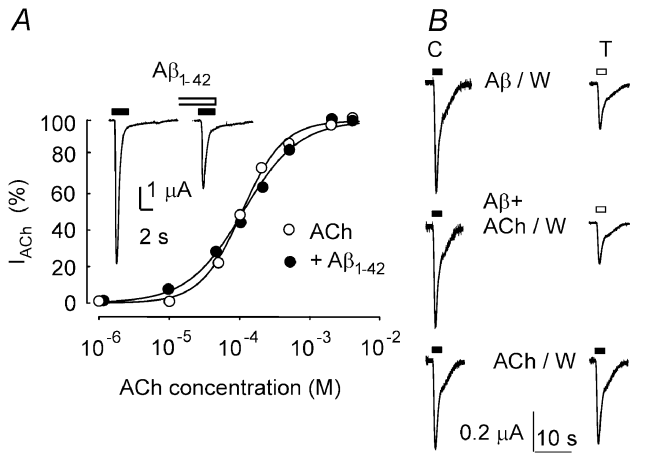

Figure 2. Non-competitive nature of $A\beta_{1–42}$-induced block of WT α7 nAChRs

A, ACh dose–response relationships obtained from 4 oocytes (1 donor) in standard solution (○) and after 180 s preincubation with 100 nM $A\beta_{1–42}$ (●). I_{ACh} was normalised to values obtained at 2 mM ACh (–2.07 μA for ○, –1.02 μA for ●). Best fitting with the Hill equation yielded: ○, $EC_{50} = 108$ μM, $n_H = 1.48$; ●, $EC_{50} = 112$ μM, $n_H = 1.20$. Inset, typical currents evoked by 2 mM ACh (filled bars) in an oocyte representative of four (2 donors) in standard solution (left) and after a preincubation with 100 nM $A\beta_{1–42}$ (right). Note the same percentage of block as in Fig. 1*A*. *B*, currents evoked by ACh (100 μM), alone (filled bar, trace labelled C) or plus $A\beta_{1–42}$ (800 nM, open bar, trace labelled T). Between trace C and T, oocytes were treated (150 s) with $A\beta_{1–42}$ (800 nM), alone (top), together with ACh (1 mM, middle) or with ACh (1 mM) alone (bottom), then washed with normal Ringer (240 s). Note the same percentage of $A\beta_{1–42}$ -induced inhibition in the T traces, independent of the presence of ACh during treatment period. All the traces were recorded from one oocyte, representative of three experiments.

The half-inhibitory concentration for $A\beta_{1–42}$ was investigated. At concentrations below 5 nM, $A\beta_{1–42}$ was not able to block α7 nAChRs, whereas at doses exceeding 100 nM there was a plateau in the inhibitory effect of the peptide, with I_{ACh} reaching 42 % of control (Fig. 1*C*). A plateau was also reported in hippocampal neurones (Liu *et al.* 2001). The apparent IC_{50} of $A\beta_{1–42}$ was 90 nM (Fig. 1*C*).

To test for the specificity of the $A\beta_{1–42}$-induced block of α7 nAChRs, we examined the effects of the peptide on the responses evoked by other neurotransmitters. In a batch of oocytes (5/1) where I_{ACh} was blocked to 44.6 ± 4.5 % of control by $A\beta_{1–42}$ (0.4 μM), the peptide was ineffective on the current evoked by AMPA (50 μM plus cyclothiazide 50 μM) in oocytes (5/2) injected with the human GluR1 subunit cDNA. In oocytes injected with mouse brain membranes (4/1), the responses evoked by AMPA (50 μM plus cyclothiazide 50 μM), kainate (200 μM), GABA (1 mM) or glycine (1 mM) were also unaffected (Fig. 1*D*). This lack of effect cannot be attributed to the structural differences between human and rodent $A\beta_{1–42}$ (3 residues), as human $A\beta_{1–42}$ is able to inhibit mouse muscle and neuronal nAChRs (Liu *et al.* 2001; Pettit *et al.* 2001; see also below). These data show that $A\beta_{1–42}$ specifically inhibits α7 nAChRs.

We next investigated the effects of the widely used, biologically inactive peptide $A\beta_{40–1}$ (100 nM). In agreement with former studies (Liu *et al.* 2001; Pettit *et al.* 2001; Dineley *et al.* 2002), this peptide was ineffective on I_{ACh}, since after a 180 s exposure to $A\beta_{40–1}$ current amplitude was 92 ± 5 % (5/2) of control (Fig. 1*C*, inset), a reduction that was not statistically significant (Student's *t* test, $P = 0.2$). At variance with former studies, we also tested the effects of peptide $A\beta_{42–1}$ (100 nM). To our surprise, it reduced I_{ACh} to 69 ± 3 % (5/2) of control values (Fig. 1*C*, inset). The block was not enhanced by raising the $A\beta_{42–1}$ concentration to 400 nM (data not shown). The most striking difference between $A\beta_{1–42}$ and $A\beta_{42–1}$ was the good reversibility of the latter. In fact, the I_{ACh} amplitude fully recovered to control values within 3 min of $A\beta_{42–1}$ removal (not shown), suggesting that the actions of $A\beta_{1–42}$ and $A\beta_{42–1}$ on α7 nAChRs are different.

The nature of the interaction between $A\beta_{1–42}$ and α7 nAChRs is controversial, as $A\beta_{1–42}$ has been reported to competitively displace α-BuTx binding (Wang *et al.* 2000*a*), whereas the inhibition of I_{ACh} appears to be non-competitive (Liu *et al.* 2001). We therefore examined how $A\beta_{1–42}$ affects the ACh dose–current response relation of human WT α7 nAChRs. In four oocytes (1 donor), during

treatment with $A\beta_{1-42}$ (100 nM), neither the EC_{50} nor n_H were significantly modified (Fig. 2*A*), in spite of the reduction of I_{ACh} amplitude, suggesting a non-competitive block of α7 nAChRs. In particular, the current evoked by a saturating ACh concentration (2 mM) was blocked to the same extent (51 ± 5 %, 4/1) as the response to 100 μM ACh (Fig. 2*A*, inset).

Given the slow onset and poor reversibility of $A\beta_{1-42}$-induced inhibition of I_{ACh}, it is possible that the competition at the ACh binding site is obscured by $A\beta_{1-42}$ dissociating too slowly to be displaced by ACh during the brief applications eliciting I_{ACh}. In order to test this hypothesis, we compared the block induced by treating the oocytes (150 s) with $A\beta_{1-42}$ alone (800 nM) or with $A\beta_{1-42}$ plus ACh (1 mM), so that competition can take place during the onset of the current inhibition. I_{ACh} (ACh concentration, 100 μM) was measured after a 240 s wash-out, which allowed for full recovery of α7 nAChRs from ACh-induced desensitisation (Fig. 2*B*, bottom). In the four oocytes tested, I_{ACh} was reduced to 44 ± 10 % of control when $A\beta_{1-42}$ was applied alone and to 46 ± 12 % when $A\beta_{1-42}$ was applied in the presence of ACh (Fig. 2*B*). We also tested whether the application of ACh (1 mM) during $A\beta_{1-42}$ wash-out could speed up I_{ACh} recovery, by accelerating the displacement of the bound peptide. Neither 10 s nor 20 s applications of ACh accelerated the recovery $A\beta_{1-42}$-inhibited current (data not shown). All these data taken together strongly support the non-competitive interaction of $A\beta_{1-42}$ with α7 nAChRs, and suggest that the mechanism of inhibition may involve the slow transition of nAChRs into a long-lived closed or blocked state.

Our data are probably explained by the reported specific binding of $A\beta_{1-42}$ to human α7 nAChRs (Wang *et al.* 2000*a,b*, 2002). However, there remains the possibility that the action of $A\beta_{1-42}$ is mediated through intracellular effectors ultimately acting on α7 nAChRs. This would be much more unlikely should we be able to demonstrate that $A\beta_{1-42}$, like many other antagonists of WT α7 nAChRs, behaves as an agonist of the mutated receptor bearing a threonine-for-leucine exchange in the M2 channel domain. We therefore studied the outcome of the exposure to $A\beta_{1-42}$ of oocytes expressing the human L248T α7 nAChR.

$A\beta_{1-42}$ is an agonist of the L248T α7 nAChR

Voltage-clamp recordings showed that brief applications (2–10 s) of $A\beta_{1-42}$ evoked currents readily blocked by methyllycaconitine (MLA, 0.2 μM) (Fig. 3*A*). Current amplitude depended on the concentration of $A\beta_{1-42}$ (Fig. 3*B* and *C*), reaching about half the amplitude of the response elicited by ACh at the saturating concentration of 100 μM (−1.1 μA; see Fucile *et al.* 2002) with a peptide concentration of 400 nM (Fig. 3*C*). In all the 15 oocytes tested (5 donors), the currents were sustained during $A\beta_{1-42}$ application, with a negligible decay observed only at high peptide concentrations (1 μM, e.g. Fig. 3*B*), as expected for this non-desensitising nAChR. Multiple $A\beta_{1-42}$ applications evoked responses of fairly constant amplitude (data not shown). These findings contrast with the observations made on rat L250T α7 nAChRs (Dineley *et al.* 2002), where responses desensitise upon multiple or prolonged applications.

The inactive $A\beta_{40-1}$ peptide (1 μM) failed to evoke responses in three out of six oocytes tested (2 donors), whereas in the other three oocytes it yielded a current whose amplitude was 12 % of the response elicited by $A\beta_{1-42}$ (1 μM) in the same oocytes. $A\beta_{42-1}$ was slightly more potent in mimicking the active peptide, eliciting currents with amplitudes which were 22 ± 15 % (6/2) of the responses elicited by $A\beta_{1-42}$ (Fig. 3*C*). However, these data confirm that current activation was largely due to a *specific* action of $A\beta_{1-42}$ on L248T α7 nAChRs, taking into account the high concentrations of peptides used in these experiments.

The action of amyloid peptides on the mutated nAChR was also investigated by performing outside-out patch-clamp recordings in oocytes expressing L248T α7 nAChRs, as determined by preliminary tests of ACh sensitivity.

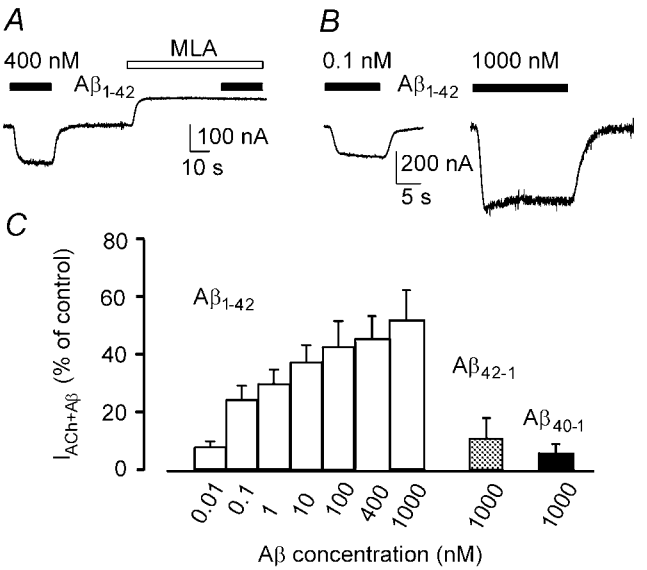

Figure 3. Activation of L248T α7 nAChRs by $A\beta_{1-42}$

A, currents activated by $A\beta_{1-42}$ (400 nM, filled bars), in an oocyte (representative of 15 oocytes, 4 donors) expressing L248T α7 nAChRs. Note the complete block by 0.2 μM MLA (~40 s preincubation, open bar). This particular response was slightly smaller than average. *B*, currents evoked by $A\beta_{1-42}$ at the indicated concentrations in two other oocytes. Note the sustained response during agonist application. *C*, histogram comparing the agonism of Aβ peptides (as indicated), normalised to the response evoked by 100 μM ACh in each oocyte. Each bar represents the mean ± S.E.M. of 6–8 oocytes (4 donors) expressing L248T α7 nAChRs.

Table 1. Single-channel properties of L248T α7 nAChR expressed in oocytes

Agonist	Conductance			Open time		
	γ_L (pS)	γ_M (pS)	γ_H (pS)	τ_{o1} (ms)	τ_{o2}(ms)	τ_{o3}(ms)
Spontaneous	41.5 ± 0.7	51.7 ± 1.7	66.2 ± 1.2	0.52 ± 0.09*	2.67 ± 0.66**	—
(19 patches)	(38 %)	(37 %)	(25 %)	(67 %)	(33 %)	
$A\beta_{1-42}$	43.9 ± 1.6	53.6 ± 0.9	67.4 ± 1.2	0.34 ± 0.03*	1.34 ± 0.14**	6.3 ± 0.8***
(9 patches)	(42 %)	(29 %)	(27 %)	(39 %)	(48 %)	(13 %)
ACh	39.1 ± 1.1	52.8 ± 1.2	64.3 ± 1.8	0.28 ± 0.01*	1.15 ± 0.05**	10.2 ± 2.2***
(10 patches)	(52 %)	(34 %)	(14 %)	(43 %)	(42 %)	(15 %)

Results are given as means ± S.E.M. (weight, %) of the indicated number of patches. Test potential, −50 mV. *, **, *** Statistically not different (one-way ANOVA, $P > 0.2$).

Table 2. Effects of $A\beta_{1-42}$ (100 nM) on whole-cell I_{ACh} in transiently transfected BOSC 23 cells

	Current amplitude			Half-decay		
Cells	I_{Ach} (nA)	Reduction (%)	Recovery (%)	$T_{0.5}$ (s)	Reduction (%)	Recovery (%)
γ-AChR	−5.1 ± 1.5 (12)	59 ± 5* (12)	66 ± 6 (8)	0.94 ± 0.23 (12)	60 ± 6** (9)	92 ± 7 (8)
ε-AChR	−0.9 ± 0.3 (3)	69 ± 3* (3)	81 (2)	1.27 ± 0.37 (3)	45 ± 1** (3)	55 (2)

Results are given as means ± S.E.M. (number of cells). Membrane potential, −70 mV; ACh concentration, 1 μM. $A\beta_{1-42}$ was applied for 80–300 s. Recovery was calculated >300 s after $A\beta_{1-42}$ washout. *, ** Statistically not different ($P > 0.15$).

Spontaneous openings of brief, MLA-sensitive channels at a frequency of 5–50 Hz were observed in all the 19 excised patches examined (17 oocytes from 9 donors), as detailed elsewhere (Fucile *et al.* 2002). It must be noted that these events differ from the well-characterised stretch-activated channels (Methfessel *et al.* 1986) both in conductance and kinetics. Application of $A\beta_{1-42}$ (1 μM) raised single-channel open probability (NP_{op}) by about 4-fold above the spontaneous background (9 patches), with MLA completely abolishing channel activity (Fig. 4*A*). In parallel experiments, ACh (0.1 μM) raised NP_{op} by about 8-fold (10 patches, data not shown).

Spontaneous and evoked unitary events showed three levels of current amplitude (e.g. Fig. 4*A*, inset), corresponding to the conductance values given in Table 1. Unitary current (*i*)–*V* relations were linear in the potential range tested (−90 to −50 mV, data not shown). More than one class of channel conductance was observed in 16 of the 19 patches examined. In each patch, the same number of conductance levels was observed for spontaneous and

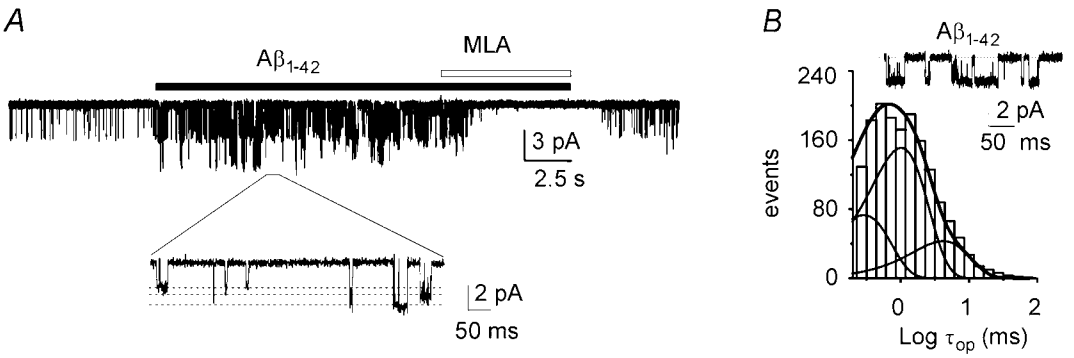

Figure 4. Single-channel properties of L248T α7 nAChRs activated by $A\beta_{1-42}$

A, spontaneous and $A\beta_{1-42}$-evoked single-channel activity, blocked by MLA (0.2 μM), in an outside-out patch from an oocyte expressing L248T α7 nAChRs. Inset, part of the trace on an expanded time scale, to show three classes of channel conductance in $A\beta_{1-42}$-evoked channel openings (γ_L = 39.7 pS, γ_M = 53.2 pS, γ_H = 67 pS). Spontaneous channels in the same patch had matching conductances. Inward currents downwards. *B*, open time distributions and sample traces for $A\beta_{1-42}$-activated channels, recorded from a different patch. Superimposed lines: best fitting exponential curves with time constants (weight): τ_{o1} = 0.29 ms (28 %), τ_{o2} = 1.02 ms (56 %), τ_{o3} = 4.11 ms (16 %), τ_{op} = 1.86 ms (n = 1689). All recordings were performed at –50 mV. $A\beta_{1-42}$ concentration, 1 μM.

evoked channels. For instance, in the nine patches exposed to $A\beta_{1-42}$, the three conductance levels were simultaneously observed in five (6 out of 10 for ACh), for both spontaneous and evoked channel (Fig. 4*A*, inset). Since no transition among the conductance levels was observed, they are likely to represent three independent gating modes of L248T α7 nAChR-channels, rather than conductance substates of a single population. This agrees with data previously described for the chick L247T α7 nAChR (Revah *et al.* 1991; Palma *et al.* 1997).

The mean open duration (τ_{op}) of spontaneous channels was 1.4 ± 0.2 ms (4068 openings from 19 patches). Upon application of $A\beta_{1-42}$, τ_{op} significantly increased to 2.1 ± 0.9 ms (11920 openings from 9 patches; one-way ANOVA, $P = 0.02$), with a distribution made up of three exponential components (Fig. 4*B*) with time constants τ_{o1}, τ_{o2} and τ_{o3} given in Table 1 (see also Fig. 4*B*). ACh-induced openings showed comparable τ_{op} values (2.7 ± 0.7 ms; 9032 openings from 10 patches; $P = 0.43$) and channel open times distribution (Table 1). Neither the opening frequency nor the τ_{op} of spontaneous channel were significantly altered when patches were exposed to $A\beta_{40-1}$ (1 μM, 4/1) (data not shown).

Muscle nAChRs are blocked by $A\beta_{1-42}$

In other experiments, we examined whether $A\beta_{1-42}$ functionally modulates the α-BuTx-sensitive mouse muscle γ- or ϵ-nAChRs, expressed in transiently transfected BOSC 23 cells. We chose this cell expression system as it yields γ- and ϵ-nAChR-channels with functional properties matching those of native muscle fibres (Grassi, 1999), while this is not the case for *Xenopus* oocytes (Kullberg *et al.* 1990). By itself, $A\beta_{1-42}$ (up to 1 μM) did not affect baseline current, nor did co-application of $A\beta_{1-42}$ together with ACh alter the current response (data not shown). However, when cells were pre-treated with $A\beta_{1-42}$ (100 nM) for 60–120 s, I_{ACh} was partially blocked (Fig. 5*A*). The effect of $A\beta_{1-42}$ developed within the first 120 s of application (Fig. 5*B*) and was not further increased by prolonged exposure to the peptide (Fig. 5*C*). The reduction of the peak current amplitude (to about 60 % of control) was accompanied by the acceleration of I_{ACh} decay and was similar for γ- and ϵ-nAChRs (Table 2), indicating that the two muscle receptors are comparably susceptible to block by $A\beta_{1-42}$.

In most cells, the amplitude of I_{ACh} did not recover to control even 10 min after $A\beta_{1-42}$ withdrawal (e.g. Fig. 5*B*), whereas $T_{0.5}$ showed a more complete recovery (Table 2). For both γ- and ϵ-nAChRs, the reduction of I_{ACh} amplitude and $T_{0.5}$ was not statistically different when changing ACh concentration in the range 0.2 to 20 μM (one-way ANOVA, $P > 0.1$). Increasing the concentration of $A\beta_{1-42}$ from 100 nM to 1 μM did not enhance the block of I_{ACh} (3 cells tested, data not shown), suggesting that maximal inhibition of I_{ACh} is already induced by the peptide at the concentration of 100 nM. As for α7 nAChRs in oocytes, the block of I_{ACh} was voltage independent in the range −30 to −90 mV (data not shown).

The effect of $A\beta_{42-1}$ on I_{ACh} was also similar to the findings in oocytes. The peptide (100 nM) reduced the amplitude of I_{ACh} to 75 % ($n = 4$, γ-nAChR) and accelerated current

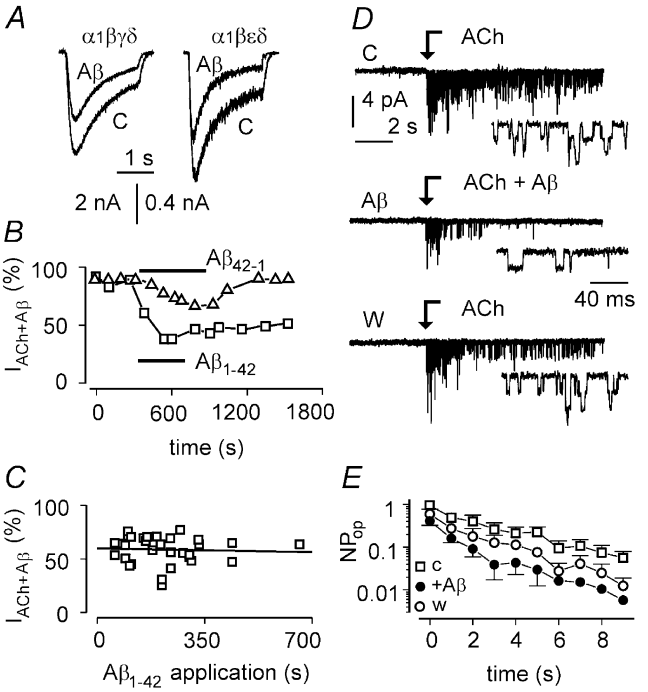

Figure 5. $A\beta_{1-42}$ blocks muscle nAChRs in transiently transfected BOSC 23 cells

A, typical inward currents evoked by ACh (1 μM) in BOSC 23 cell expressing γ-AChR (left) or ϵ-AChR (right), before (C) or after (Aβ) 120 s of application of $A\beta_{1-42}$ (100 nM). Note the accelerated decay of I_{ACh} during $A\beta_{1-42}$ application. *B*, time course of I_{ACh} block by $A\beta_{1-42}$ or $A\beta_{42-1}$ (both 100 nM) in two different cells expressing γ-AChR. Bars, Aβ applications. Note the lack of recovery 15 min after $A\beta_{1-42}$ wash-out, as compared to the prompt recovery upon $A\beta_{42-1}$ removal. ACh concentration, 1 μM. I_{ACh} normalised to control current amplitude. *C*, plot of I_{ACh} amplitude (normalised to the control in each cell) *vs.* the duration of $A\beta_{1-42}$ (100 nM) application. The continuous line represents the linear regression of the data, with a slope of −0.005 % s^{-1} ($R = 0.05$), indicating that the block of I_{ACh} is independent of the duration of $A\beta_{1-42}$ application. All the data obtained for γ-AChR, irrespective of ACh concentration (0.2–20 μM), are included in this plot. *D*, activation of γ-nAChR-channels by ACh (1 μM) in an outside-out patch, before (trace C), during (Aβ) and after (W) the application of $A\beta_{1-42}$. τ_{cl}, 26 ms (C), 184 ms (Aβ), 60 ms (W). $A\beta_{1-42}$ (100 nM) application began 60 s before recording trace Aβ, terminated 5 min before recording trace W. Traces were filtered at 200 Hz for display purposes. Insets, expanded traces beginning 750 ms after ACh application (filter, 1 kHz). Single-channel conductance (35.7 pS) and open duration (3.5 ms) were not affected by $A\beta_{1-42}$. *E*, plot of the average NP_{op}, normalised to the control value of each patch, in 5 outside-out patches, measured over 1-s intervals during the first 10 s of ACh (1 μM) application. Data were sampled before (□), in the continuous presence of $A\beta_{1-42}$ (30–120 s preincubation, (●), or 30–120 s after $A\beta_{1-42}$ wash-out (○).

decay. This reduced block was reversible within min of $A\beta_{42-1}$ wash-out (e.g. Fig. 5*B*), at variance with the effect of $A\beta_{1-42}$.

The effects of $A\beta_{1-42}$ (100 nM) on the single-channel properties of muscle nAChRs were investigated in five outside-out patches from cells expressing γ-nAChRs. Neither the conductance (39.7 ± 1.4 pS) nor the τ_{op} (3.2 ± 0.5 ms) of the events evoked by ACh (1 μM) were affected by applying $A\beta_{1-42}$ (100 nM) for 60–120 s. After this pre-treatment, application of ACh in the continuous presence of the peptide elicited single-channel openings with a conductance of 40.5 ± 1.5 pS and τ_{op} of 3.1 ± 0.5 ms (e.g. Fig. 5*D*, inset). During long lasting ACh applications, channel opening frequency markedly decreased, while channel conductance and τ_{op} remained stable. Channel closed time (τ_{cl}) increased from 2- to 10-fold within 30–60 s of $A\beta_{1-42}$ exposure (e.g. Fig. 5*D*). Given the non-stationary behaviour of channel activity in these patches, NP_{op} was measured over 1-s intervals during the first 10 s of ACh application. In good agreement with whole-cell data, after 30–120 s of $A\beta_{1-42}$ application, NP_{op} was reduced to about 45 % control and the rate of NP_{op} decrease was accelerated by about 50 % (Fig. 5*E*), indicating that $A\beta_{1-42}$ promotes the block of ACh-evoked channels. The effects of $A\beta_{1-42}$ were only partially reversible by 30 s wash-out (see Fig. 5*E*). Longer washes (>120 s) could be performed in only two patches, and yielded almost full recovery.

To examine whether $A\beta_{1-42}$ modulates muscle nAChRs *via* indirect pathways, the peptide was applied to the extra-patch membrane while recording γ-nAChR single-channel activity under cell-attached conditions. In the four patches examined, the unitary channel conductance remained unchanged during $A\beta_{1-42}$ applications lasting 2–6 min (33 pS, data not shown). Channel opening frequency reversibly decreased to 30 % of control in one patch; channel mean open time decreased to 85 % in another patch. Taken together with the results of outside-out recordings, these data are consistent with the hypothesis that $A\beta_{1-42}$ exerts its effects by directly binding to the nAChR molecule.

DISCUSSION

A high-affinity association of the amyloid peptide $A\beta_{1-42}$ with α7 nAChRs has recently been observed in amyloid plaques and in the neurons of AD patients (Wang *et al.* 2000*a*,*b*, 2002; Nagele *et al.* 2002). However, the modulation of the α7 nAChR function has only been described in chick and rodent preparations (Liu *et al.* 2001; Pettit *et al.* 2001; Dineley *et al.* 2002; Tozaki *et al.* 2002). In this paper, we give evidence that $A\beta_{1-42}$ is able to functionally block the human neuronal α7 nAChR, in a poorly reversible manner, with a potency comparable with that previously described for native and reconstituted rat preparations (Liu *et al.* 2001; Pettit *et al.* 2001; Dineley *et al.* 2002). Moreover, mouse muscle γ- and ϵ-nAChRs, other types of α-BuTx-sensitive nAChR, were blocked by $A\beta_{1-42}$ in a manner rather similar to the block of α7 nAChRs, the main difference being that $A\beta_{1-42}$ accelerates the rate of current decay for muscle but not neuronal nAChRs, as already described in rat hippocampal cultures (Liu *et al.* 2001). Thus, blockade of α-BuTx-sensitive nAChRs by $A\beta_{1-42}$ appears to be a rather general property, although other studies have found it to be fully reversible (Liu *et al.* 2001; Pettit *et al.* 2001).

It has been reported that picomolar concentrations of $A\beta_{1-42}$ elicit current responses from oocytes expressing rat WT α7 nAChRs (Dineley *et al.* 2002), even though no activation was seen in rat hippocampal neurones exposed to similar concentrations of $A\beta_{1-42}$ (Liu *et al.* 2001). In our hands, human WT α7 nAChRs were not activated by $A\beta_{1-42}$ over a wide range of concentrations, although, in parallel experiments, the L248T mutant α7 receptor did respond to the peptide. Thus, $A\beta_{1-42}$-induced activation of WT α7 nAChRs appears to be strongly dependent on the receptor type, the cell system, or the experimental procedure.

It might be argued that our data lack specificity, as the inverse peptide, $A\beta_{42-1}$, does inhibit I_{ACh}. To the best of our knowledge, this is the first report of the biological effects of $A\beta_{42-1}$. In particular, in the papers investigating the interaction between $A\beta_{1-42}$ and α7 nAChRs, the only peptide used as a control was $A\beta_{40-1}$ (Wang *et al.* 2000*a*,*b*; Liu *et al.* 2001; Pettit *et al.* 2001; Dineley *et al.* 2002). In our hands, $A\beta_{40-1}$ is ineffective in inhibiting WT α7 nAChRs, and only marginally capable of activating L248T mutant α7 nAChRs when used at very high concentrations (1 μM). Comparably small effects of $A\beta_{40-1}$ have been observed when measuring α-BuTx binding to α7 nAChRs (Wang *et al.* 2000*a*) or current block (Liu *et al.* 2001), and were considered negligible. Thus, the effects of $A\beta_{1-42}$ on muscle and neuronal nAChRs reported here can be claimed to be as specific as those previously reported. Two questions remain open: what causes the reverse peptide $A\beta_{42-1}$ to be active, and why is $A\beta_{42-1}$ effective while $A\beta_{40-1}$ is not. The hypothesis that the effects are due to peptide contaminants is rather unlikely, since we used peptides of different origin and in different solvents. It must be noted that similarities in the neurotoxic action of $A\beta_{1-40}$ and $A\beta_{40-1}$ have been reported (Giordano *et al.* 1994), indicating that reverse peptides are not entirely biologically inactive. It has been shown that the smaller fragment $A\beta_{12-28}$ is able to mimic the action of the $A\beta_{1-42}$ peptide on muscle and WT α7 nAChRs (Wang *et al.* 2000*b*; Pettit *et al.* 2001; authors' unpublished observations), indicating that a binding epitope for nAChRs resides in this peptide region, which comprises an α-helix and a 'kink' region (Coles *et al.* 1998). That the binding epitope is conserved in the reverse

peptide is quite unlikely, but it might be possible that the reverse peptide contains another binding site for nAChRs, causing a weaker block. In line with this hypothesis, the block by $A\beta_{42-1}$ is fully reversible, while the effect of $A\beta_{1-42}$ is not, suggesting differential interactions of the two peptides with nAChRs. It may be speculated that the two very hydrophobic terminal amino acids (an isoleucine and an alanine) present in $A\beta_{42-1}$, but not in $A\beta_{40-1}$, favour the interaction of the longer peptide with the cell membrane, thus enhancing the probability of an interaction with nAChRs. Understanding the interaction between $A\beta_{42-1}$ and nAChRs is, however, beyond the scope of this paper, especially because the effect of $A\beta_{1-42}$, being stronger than that of $A\beta_{42-1}$, is likely to be biologically relevant.

In agreement with other studies (Liu *et al.* 2001; Pettit *et al.* 2001; Dineley *et al.* 2002), we report that the $A\beta_{1-42}$-induced block of I_{ACh} requires a few minutes of preincubation, both for α7-expressing oocytes and for γ- and ϵ-nAChR-expressing BOSC 23 cells. This might suggest the involvement of pathways mediated by second messengers. Several pieces of evidence argue against this hypothesis. First, in BOSC 23 cells, the reduced amplitude and accelerated decay of whole-cell I_{ACh} upon application of $A\beta_{1-42}$ matches the reduced NP_{op} and faster desensitisation of γ-nAChR-channels observed in cell-free outside-out patches, where the cytosolic components are lost. Second, cell-attached recordings in intact cells, with a fully preserved cytoplasmic environment, failed to reveal any indirect effect of $A\beta_{1-42}$ on γ-nAChR-channel activity. Third, $A\beta_{1-42}$ behaves as an agonist of the L248T mutant α7 nAChR, as do many other α7 nAChR antagonists whose direct actions on nAChRs are very firmly established. Fourth, this agonist action is also seen in excised patches, again ruling out the requirement for cytoplasmic components. It is noteworthy, however, that the agonist action of $A\beta_{1-42}$ on L248T nAChRs is rapid, both on intact oocytes and in outside-out patches (our data and Dineley *et al.* 2002). It is possible that a simple gating process activates the mutant α7 nAChR, whereas blockade of WT α7 and muscle receptors requires the slow stabilisation of an inactive state. The poor reversibility of the inhibition is also compatible with the hypothesis of $A\beta_{1-42}$ driving the nAChRs into a long-lived closed (or blocked) conformation.

The significance of this interaction between $A\beta_{1-42}$ and α7 nAChRs for the aetiology or the pathogenesis of AD is unclear. Recent work shows a preferential accumulation of $A\beta_{1-42}$ in neurons expressing α7 nAChRs (Wang *et al.* 2000*a*,*b*, 2002) and evidence has been provided that intracellular accumulation of $A\beta_{1-42}$ may be facilitated by α7 nAChRs (Nagele *et al.* 2002), thus implying a relevant physio-pathological role for the interaction. This raises the possibility that the binding of $A\beta_{1-42}$ to muscle ϵ-nAChRs might be related to the initiation of plaque deposition in IBM and/or in the muscles of AD patients, which show an increased content of $A\beta_{1-42}$ (Kuo *et al.* 2000*b*). The functional modulation of muscle nAChRs by $A\beta_{1-42}$ strengthens the similarity between AD and IBM, further suggesting that the two diseases share at least some pathogenic mechanisms.

The question remains whether the observed $A\beta_{1-42}$-induced nAChR functional changes affect synaptic transmission. We and others (Pettit *et al.* 2001; Dineley *et al.* 2002) have shown that $A\beta_{1-42}$ affects I_{ACh} with an IC_{50} around 100 nM (that is, about 450 ng ml^{-1}), although an IC_{50} of about 7.5 nM has been described for rat hippocampal neurones (Liu *et al.* 2001). The concentrations of $A\beta_{1-42}$ in the plasma and cerebrospinal fluid of control and AD humans are uncertain, reported values ranging between 0.04 ng ml^{-1} (i.e. 0.01 nM, Mehta *et al.* 2000) and 20 ng ml^{-1} (i.e. 5 nM, Kuo *et al.* 2000*a*). These values are lower than the observed IC_{50}, but functional modulation of α7 nAChR *in vivo* might ensue because the neurones are tonically exposed to $A\beta_{1-42}$, that is, for times much longer than have been tested in experimental studies.

A possible link between $A\beta_{1-42}$ binding to α7 nAChRs and cognitive impairments in AD was recently suggested by a paper (Dineley *et al.* 2001) showing that $A\beta_{1-42}$ is able to promote MAP kinase activation by inducing Ca^{2+} influx through α7 nAChRs, thereby interfering with long term potentiation processes. That study, however, was conducted in mice heterozygous for the mutant L250T α7 nAChR and we show here that $A\beta_{1-42}$ does not activate the human WT α7 nAChR. The fact that *human* WT α7 nAChRs is not activatable by $A\beta_{1-42}$ rules out the likelihood that memory loss in AD is caused by the suggested mechanism. Nevertheless, the activation of L248T α7 nAChRs by $A\beta_{1-42}$ raises the possibility of a correlation between genetic variations of α7 nAChRs and AD. The hypothesis that an allelic variant, a 2 bp deletion, of the partially duplicated human gene encoding the α7 subunit induces susceptibility to AD has recently been tested and dismissed (Liou *et al.* 2001). To our knowledge, other mutations have not been investigated.

In conclusion, we give evidence that $A\beta_{1-42}$ alters the gating of α-BuTx-sensitive nAChRs, blocking human WT α7 nAChRs and mouse muscle nAChRs, while activating the human mutant L248T α7 nAChR. The functional impairment of nAChRs might be responsible, at least in part, for the cognitive deficits known to appear well before plaque formation both in mouse models (Moechars *et al.* 1999) and in AD patients (for review, see Neve & Robakis, 1998; Smith, 2002). The loss of synaptic input to cortical areas might underlie AD progression from the medial temporal lobe to the whole cerebral cortex (Smith, 2002). Further research should elucidate this point.

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